Draft NTP Technical Report TR 583

Bromodichloroacetic acid

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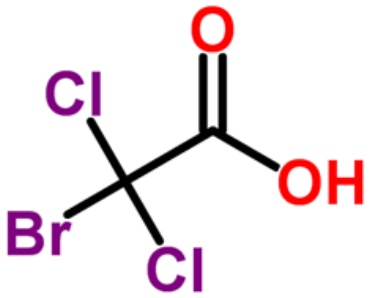
NTP Technical Reports Peer Review Meeting
May 22, 2014

Nomination



- Nominated by:
  - United States Environmental Protection Agency
  - American Water Works Association Research Foundation

- Nomination based on:
  - Widespread exposure to bromodichloroacetic acid through drinking water
  - Structurally similar to other haloacetic acids that are carcinogenic in rodents
  - Lack of toxicity and carcinogenicity data

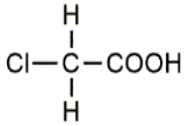
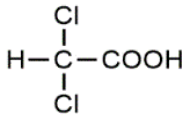
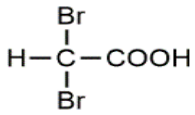
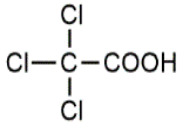
Bromodichloroacetic acid: Use and Exposure

- Water disinfection by-product: One of the haloacetic acids
- No known commercial use
- Not regulated by EPA and often not measured in drinking water
- Bromodichloroacetic acid is 1-20% of the total haloacetic acids Weinberg et al. (2002)
- Only ≈2% of water suppliers provide information on bromodichloroacetic acid (Environmental Working Group)
- Highest levels reported are ≈ 17 ug/L (Weinberg et al and Environmental Working Group)

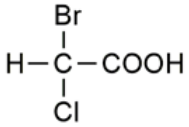
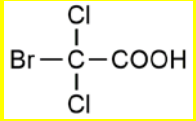
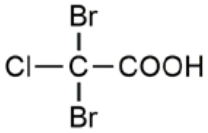
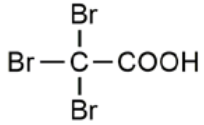
NTP and Water Disinfection By-Products

- A number of classes of water disinfection by-products have been nominated to NTP
  - Halomethanes
  - Haloacetic acids
  - Oxyhalides
- Focus has been on chlorinated, brominated and mixed chloro/bromo byproducts

Carcinogenicity Studies of Regulated Haloacetic acids

Chemical	Study	Carcinogenicity
Monochloroacetic acid 	NTP (TR-396)	Rats: no evidence Mice: no evidence
Monobromoacetic acid	*Not tested by the NTP* *No studies in the published literature*	
Dichloroacetic acid 	Carter et al. (2003); Pereira (1996); DeAngelo et al. (1991) DeAngelo et al. (1996) NTP (GMM-11) FVB Tg.AC hemizygous mice B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice	B6C3F1 mouse - Liver tumors (males and females) F344 Rat - Liver tumors (males) FVB Tg.AC hemizygous mice: squamous cell papillomas (males and females) alveolar/bronchiolar tumors (males and females) B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice: no evidence
Dibromoacetic acid 	NTP (TR-537)	Rats: malignant mesothelioma (males) and mononuclear cell leukemia (females) Mice: hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma (males and females); alveolar/bronchiolar adenoma (males)
Trichloroacetic acid 	Herren-Freund et al. (1987); DeAngelo and Daniel (1990) DeAngelo et al. (1997)	B6C3F1 mouse - Liver tumors (males) F344 rat - no evidence (males only)

Carcinogenicity Studies of Unregulated Haloacetic acids

Chemical	Study	Carcinogenicity
Bromochloroacetic acid 	NTP (TR-549)	Rats: malignant mesothelioma (males); large intestine adenomas (males and females); mammary gland (females) Mice: hepatocellular adenoma, carcinoma (males and females); hepatoblastoma (males)
Bromodichloroacetic acid 	NTP (TR-583)	Under review
Chlorodibromoacetic acid 	Not tested by NTP No studies in the published literature	
Tribromoacetic acid 	Not tested by NTP No studies in the published literature	

Experimental Design

- Genotoxicity studies: in vitro and in vivo (mice)
- Toxicity/Carcinogenicity studies
  - F344/N and F344/NTac rats, and B6C3F1/N mice
  - Bromodichloroacetic acid exposure through drinking water:
    - 2- Week Study
      - Rats (F344/N) and Mice (n=5): 0, 62.5, 125, 250, 500 and 1000 mg/L
    - 3-Month Study
      - Rats (F344/N) and mice (n=10): 0, 62.5, 125, 250, 500 and 1000 mg/L
    - 2-Year study
      - Rats (**F344/NTac**) and mice (n=50**)**: 0, 250, 500 and 1000 mg/L
        - 6 month interim (n=8)
        - 13-month (rats) or 14-month (mice) interim (n=8)

Genetic Toxicity

- Bromodichloroacetic acid was **mutagenic** in *Salmonella typhimurium* strains TA97, TA98, and TA100, and in *Escherichia coli* WP2 *uvrA* without S9 activation, and in the *E. coli* strain with S9 activation

- **Equivocal** results were observed in *Salmonella typhimurium* strains TA97, TA98, and TA100 with S9 activation

- **No increases** in micronucleated erythrocytes were observed in mice following a 3-month exposure in drinking water

2-Week Study of Bromodichloroacetic Acid in F344/N Rats and B6C3F1/N mice

- No effects on body or organ weights
- No clinical findings associated with exposure

3-Month Studies of Bromodichloroacetic Acid in F344/N Rats and B6C3F1/N mice

- No chemical-related difference in body weight or body weight gains
- No chemical-related gross or histopathology findings
- Minor changes (< 15%) in liver and kidney weights (rats and mice) at the high exposure concentrations

Dose Selection for 2-Year Studies (Rats and Mice)

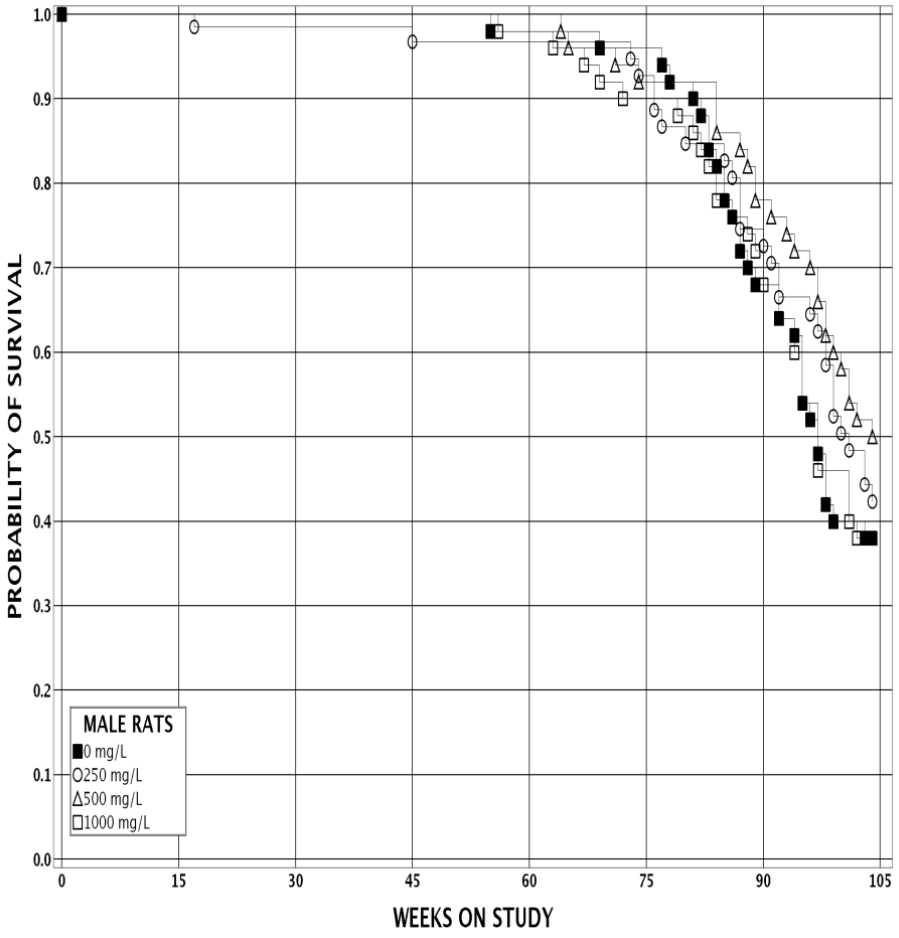
- Based on the limited effects of bromodichloroacetic acid in the 3-month studies, the exposure concentrations selected were 0, 250, 500 and 1,000 mg/L

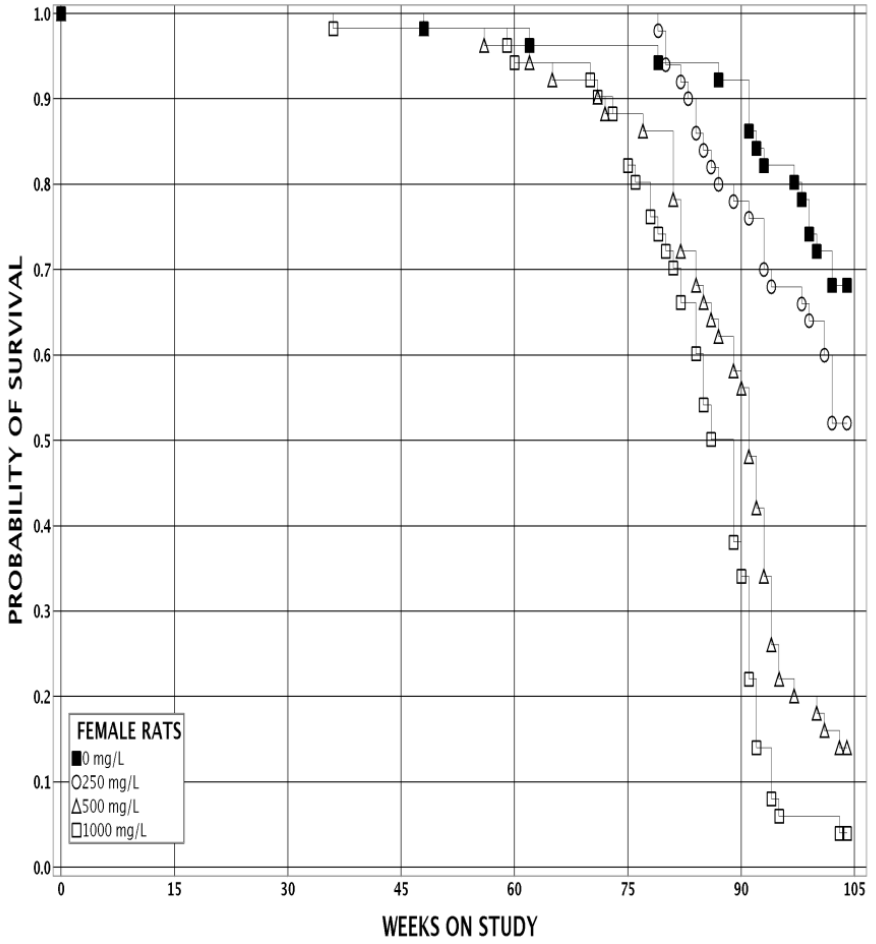
- Average Daily Intake
  - Rats
    - Males: 11, 21, and 43 mg/kg/d
    - Females: 13, 28, and 57 mg/kg/d
  - Mice
    - Males: 23, 52, and 108 mg/kg/d
    - Females: 17, 34, and 68 mg/kg/d

Challenges using the F344/NTac Rat

- Several tumor types with low/rare background incidence in controls were observed in the exposed animals
- Only one other NTP carcinogenicity study used the F344/NTac rats
- In these cases, our past experience with a related strain (F344/N) was taken into consideration
- In cases of low incidence or rare tumors, factors in addition to statistical analysis were considered

Kaplan-Meier Survival Curves for F344/NTac Rats Exposed to Bromodichloroacetic Acid in Drinking Water for 2 Years





10% decrease in body weights in 1,000 mg/L females starting at week 13
10% decrease in body weights in 1,000 mg/L males starting at week 89

Malignant Mesotheliomas in Male F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
13-Month Interim Evaluation				
Number Examined	8	8	8	8
Malignant Mesotheliomas (includes bilateral)	0	0	1	5*
2-Year Study				
Malignant Mesotheliomas (Multiple organs)[a]	1*	12*	18*	37*

* Significantly different (P≤0.05) from the control group by the Poly-3 test; when in controls indicates significant trend

Neoplastic and Nonneoplastic Mammary Gland Lesions in F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Females				
Hyperplasia[a]	0	4*(1.3)	2* (1.5)	10* (1.2)
Fibroadenoma (includes multiples)	28*	47*	47*	39*
Adenoma	1	2	3	1
Carcinoma	0*	1	3	8*
Males				
Hyperplasia[a]	6 (1.2)	1* (1.0)	2 (1.0)	1 (1.0)
Fibroadenoma	0	2	3	1

***** Significantly different (P≤0.05) from the control group by the Poly-3 test; in control indicates significant trend
[a] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TGF-β signaling in mammary gland carcinomas resulting from 2-year BDCA exposure in F344/NTac rats

- Differentiate between mammary carcinomas in control and BDCA treated groups
- Laser Capture Microdissection of age-matched normal mammary gland epithelium, and mammary carcinomas from BDCA treated and unexposed rats
- PCR array specific for mammary carcinogenesis
- 8/84 genes were unique to mammary carcinomas from BDCA exposed rats
- Majority of these genes (*Mmp9*, *Mmp2*, *Id1*, *Vegfa*, and *Thbs1*) were associated with TGF-β signaling with effects on matrix remodeling, EMT, tumor invasion and progression

Incidences of Neoplasms of the Skin in Male F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Squamous Cell Papilloma	3	1	0	1
Keratoacanthoma (includes multiples)	7	3	10	15*
Basal Cell Adenoma	0	0	4	4
Basal Cell Carcinoma	0	0	0	1
Squamous Cell Carcinoma	0	1	1	0
Sebaceous Gland Adenoma	0	2	2	2
Combined Epithelial skin neoplasms	9*	7	15	21*
Subcutaneous Tissue Fibroma	4*	6	10	15*

***** Significantly different (P≤0.05) from the control group by the Poly-3 test; when in control, indicates significant trend

Incidences of Neoplasms of the Brain in Male F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Original Evaluation				
Glioma	0	1	2	2
Oligodendroglioma	0	0	1	1
Glioma or Oligodendroglioma	0	1	3	3
Original and Extended Evaluation				
Glioma	1	1	2	2
Oligodendroglioma	0	0	2	1
Glioma or Oligodendroglioma	1	1	4	3

Incidences of Gliomas and Oligodendrogliomas of the Brain in Female F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Glioma	1	0	2	0
Oligodendroglioma	0	0	1	1
Glioma or Oligodendroglioma	1	0	3	1

Note: No additional tumors were observed in the extended evaluation

Gliomas and Oligodendrogliomas of the Brain in F344/NTac Rats

- Males
  - **Some Evidence**
    - Incidence of gliomas and oligodendrogliomas: 1/50, 1/50, 4/50 and 3/50
    - 2013 F344/N historical controls: No studies with more than 1/50 in the controls
- Females
  - **Some Evidence**
    - Incidence of gliomas and oligodendrogliomas:1/50, 0/50, 3/50 and 1/50
    - 2013 F344/N historical controls: No studies with more than 1/50 in the controls

Incidences of Neoplastic and Nonneoplastic Lesions of the Oral Cavity in Male F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Epithelial Hyperplasia[a]	0	0	1 (2.0)	2 (2.5)
Squamous Cell Papilloma	0	0	2	2
Squamous Cell Carcinoma	1	0	1	1
Papilloma or Carcinoma	1	0	3	3

[a] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Squamous Cell Papillomas and Carcinomas of the Oral Cavity

- Males
  - **Some Evidence**
    - 3 neoplasms in the two highest exposure groups and 1 in the concurrent controls
    - An association with hyperplasia of the lingual epithelium
    - The oral cavity as a portal of entry
    - 2013 F344/N historical controls: No studies with more than 1/50 in the controls

Incidences of Adenomas of the Large Intestine in Male F344/NTac Rats

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Cecum	0	0	1	0
Colon	0	0	1	1
Rectum	0	0	0	1
Cecum, Colon or Rectum	0	0	2	2

Adenomas of the Large Intestine

- Males
  - **Equivocal Evidence**
    - Incidence of adenomas of large intestine: 0/50, 0/50, 2/50 and 2/50
    - 2013 F344/N historical controls: No studies with more than 1/50 in the controls
    - Bromochloroacetic acid increased incidence of adenomas of the large intestine

Non-neoplastic lesions in F344/NTac Rats exposed to Bromodichloroacetic Acid

- Liver
  - Males: Eosinophilic focus
  - Females: Eosinophilic focus and hematopoietic cell proliferation
- Spleen
  - Females: Hematopoietic cell proliferation
- Bone Marrow
  - Males: Angiectasis and hyperplasia
  - Females: Angiectasis and hyperplasia

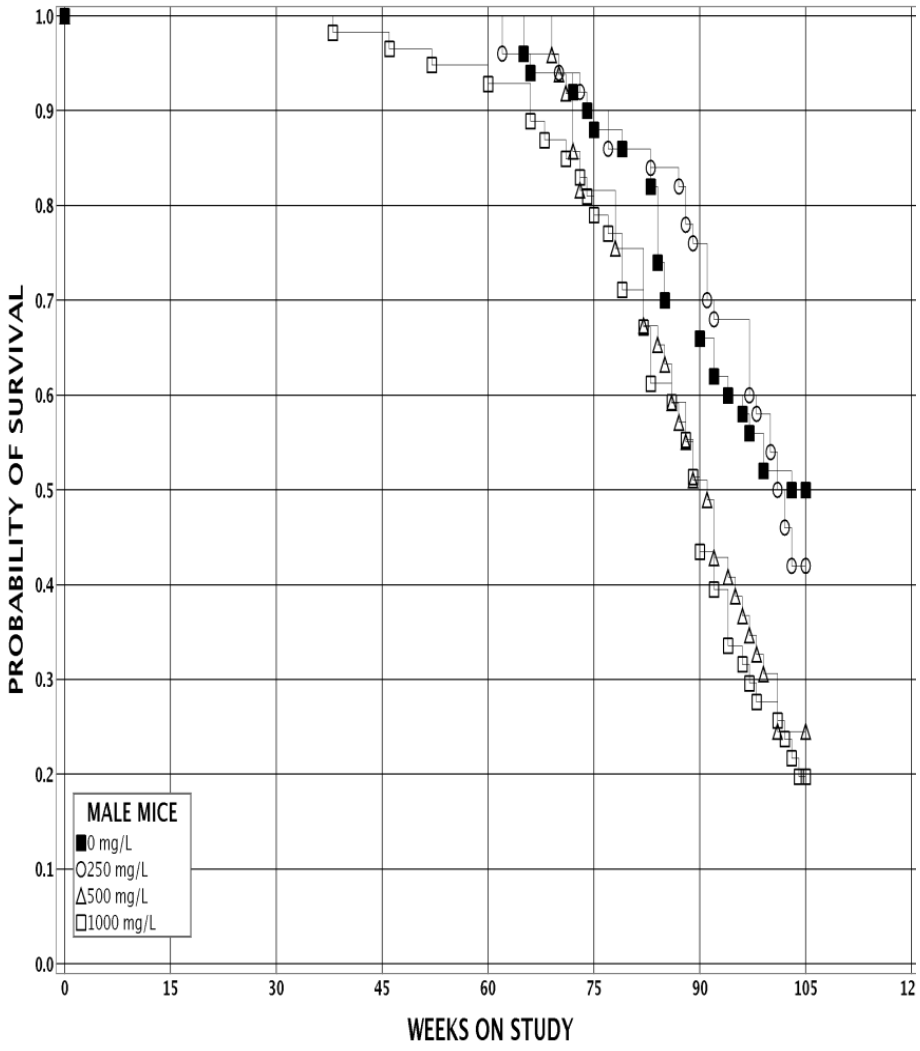
Summary of Carcinogenic Effects of Bromodichloroacetic Acid in Male F344/NTac Rats

- ***Clear evidence of carcinogenic activity***:
  - Increased incidences of malignant mesothelioma
  - Combined incidences of epithelial tumors of the skin (squamous cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma)
- Related to exposure (some evidence):
  - Increased incidences of glioma or oligodendroglioma (combined) of the brain
  - Increased incidences of squamous cell papilloma or squamous cell carcinoma of the oral cavity (oral mucosa or tongue)
  - Increased incidences of subcutaneous fibromas
- May have been related to exposure (equivocal evidence):
  - Occurrences of adenoma of the large intestine
  - Occurrences fibroadenoma of the mammary gland

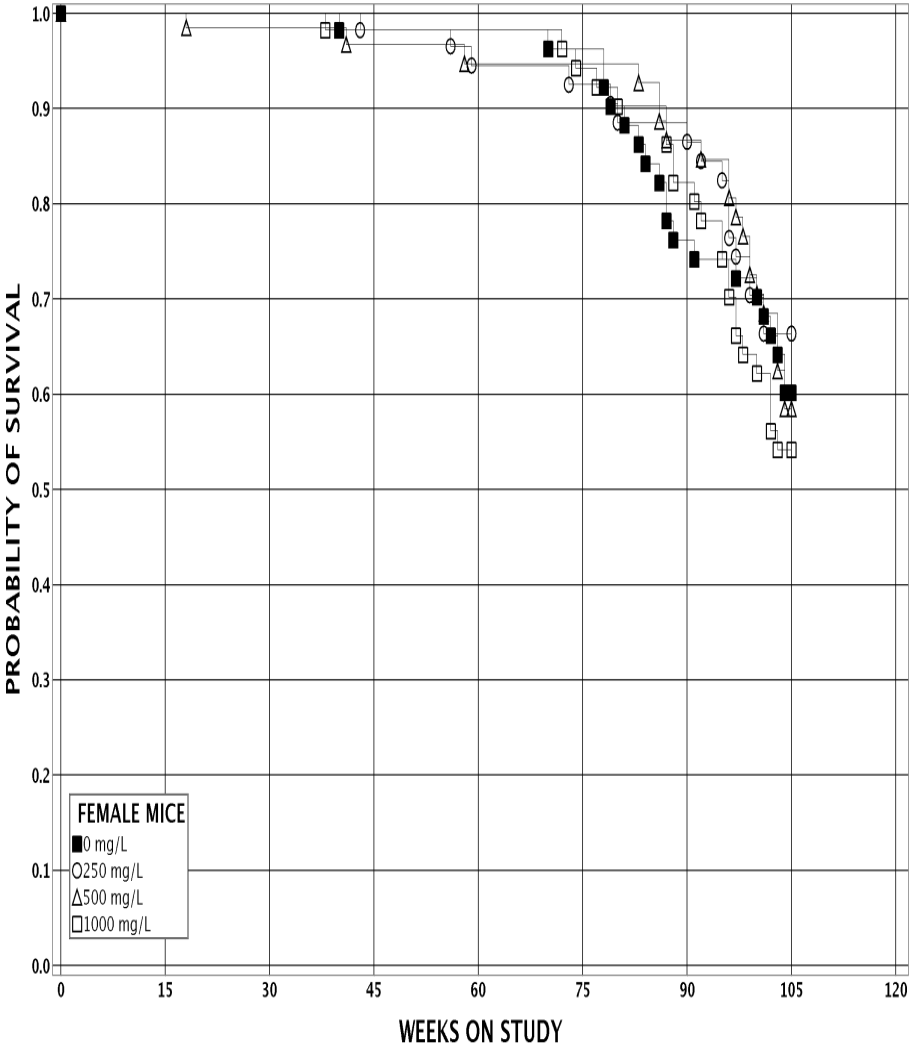
Summary of Carcinogenic Effects of Bromodichloracetic Acid in Female F344/NTac Rats

- ***Clear evidence of carcinogenic activity***:
  - Increased incidences of fibroadenoma and carcinoma of the mammary gland
- Related to exposure (some evidence):
  - Increased incidences of glioma or oligodendroglioma (combined) of the brain

Kaplan-Meier Survival Curves for B6C3F1/N Mice Exposed to Bromodichloroacetic Acid in Drinking Water for 2 Years

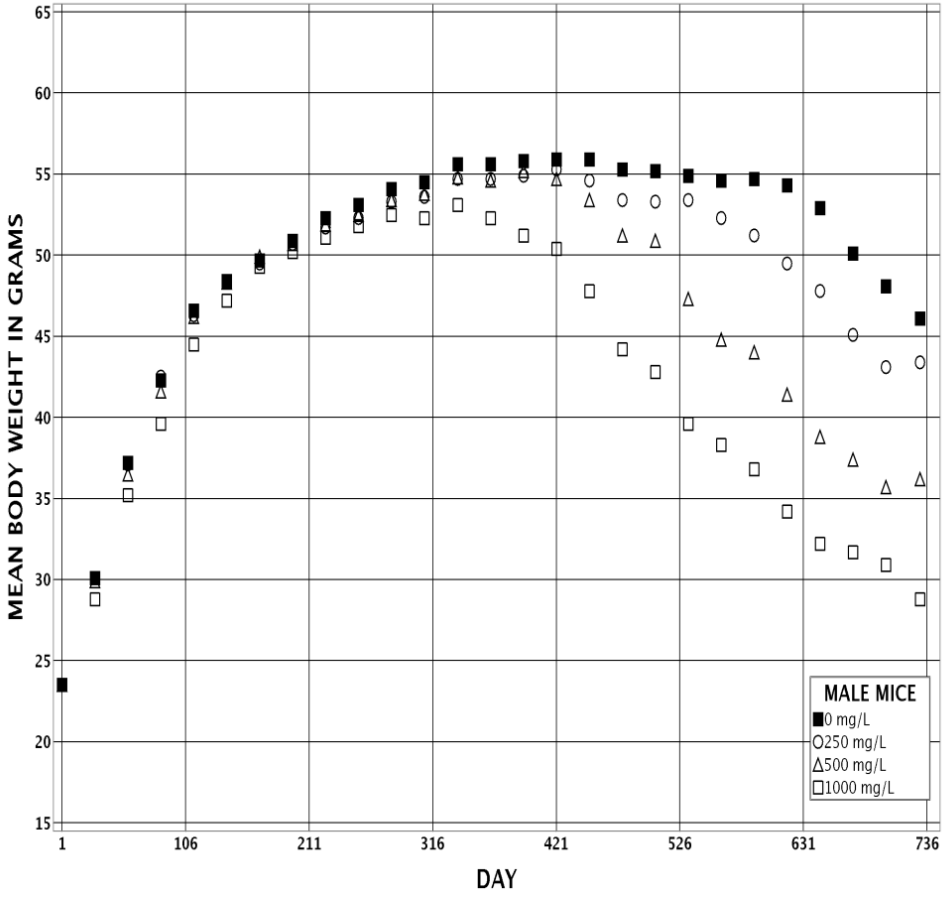


Male Mice

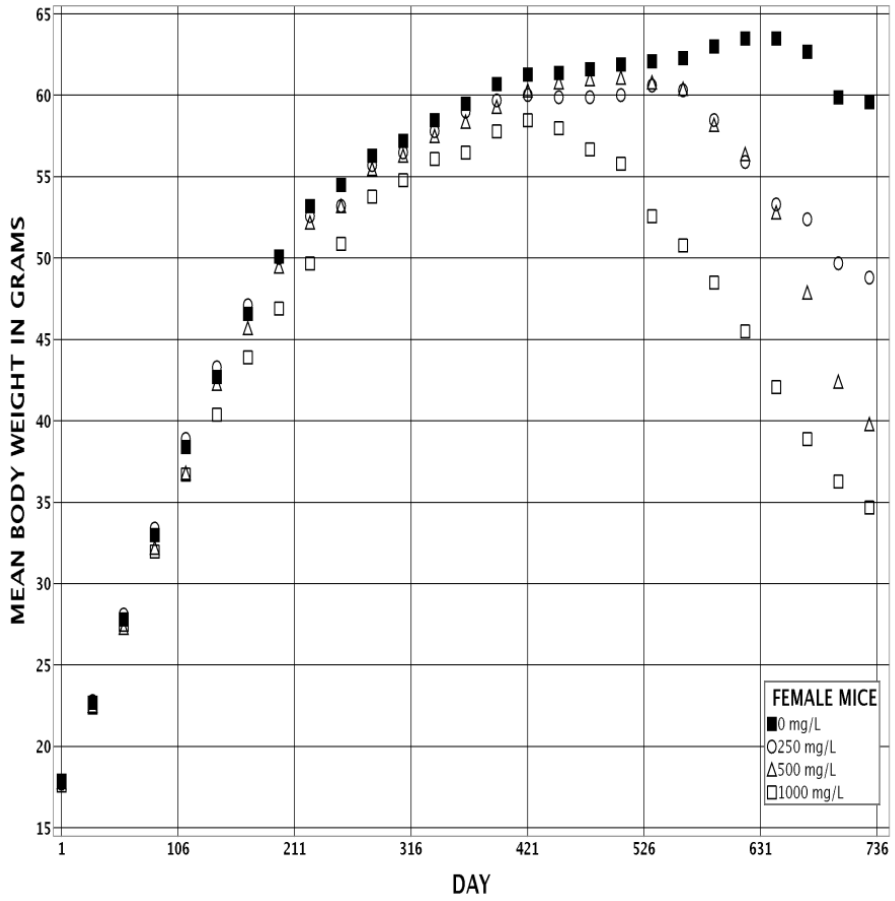


Female Mice

Effects on Body Weights for B6C3F1/N Mice Exposed to Bromodichloroacetic Acid in Drinking Water for 2 Years



Male Mice



Female Mice

Incidence of Neoplastic and Nonneoplastic lesions in the Liver of Male B6C3F1/N Mice

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
14-Month Interim Evaluation				
Focus of Cellular Alteration, Atypical[a]	1	2	4	6*
Hepatocellular Adenoma	0	2	1	1
Hepatoblastoma	0	0	0	1
2-Year Study				
Focus of Cellular Alteration, Atypical	0	19*	42*	43*
Hepatocellular Adenoma	39	41	42	40
Hepatocellular Carcinoma[b]	12*	22*	27*	39*
Hepatoblastoma[c]	4*	24*	40*	34*

***** Significantly different (P≤0.05) from the control group by the Poly-3 test; when in control, indicates significant trend

[b] Historical incidence for drinking water studies: 20/98 (20.4% ± 2.9%), range 18%-22%;

[c] Historical incidence for drinking water studies: 1/98 (1.0% ± 1.44%), range 0%-2%;

Incidence of Neoplastic and Nonneoplastic Lesions in the Liver of Female B6C3F1/N Mice

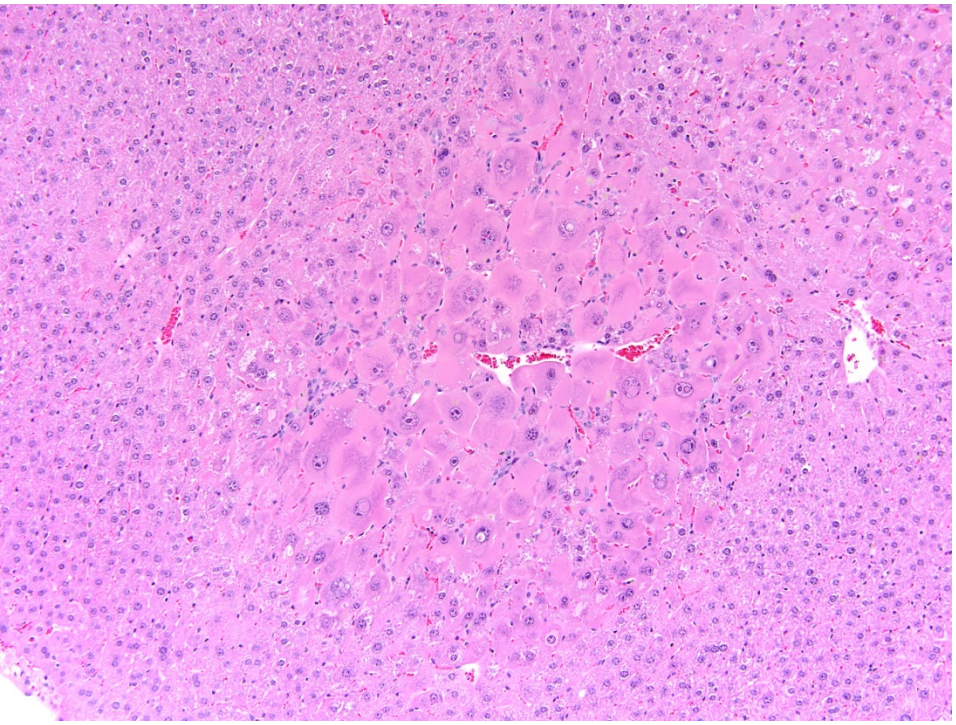
	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
14-Month Interim Evaluation				
Focus of Cellular Alteration, Atypical	0	2	3	4*
Hepatocellular Adenoma	0	0	1	1
2-Year Study				
Focus of Cellular Alteration, Atypical	0	2	6*	16*
Eosinophilic Focus	22	33*	38*	40*
Hepatocellular Adenoma	33*	42*	42*	44*
Hepatocellular Carcinoma[b]	9*	17	22*	26*
Hepatoblastoma[c]	0*	1	4	6*

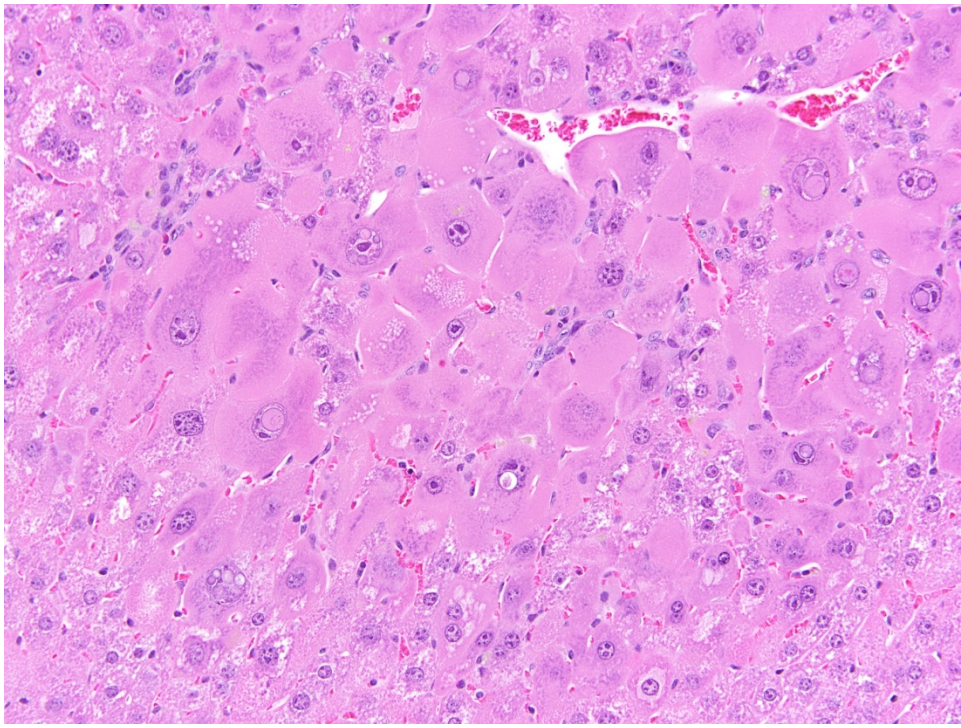
* Significantly different (P≤0.05) from the control group by the Poly-3 test; when in controls, indicates significant trend

[b] Historical incidence for drinking water studies: 38/100 (38.0% ± 19.8%), range 24%-52%;

[c] Historical incidence for drinking water studies: 2/100 (2.0% ± 2.8%), range 0%-4%;

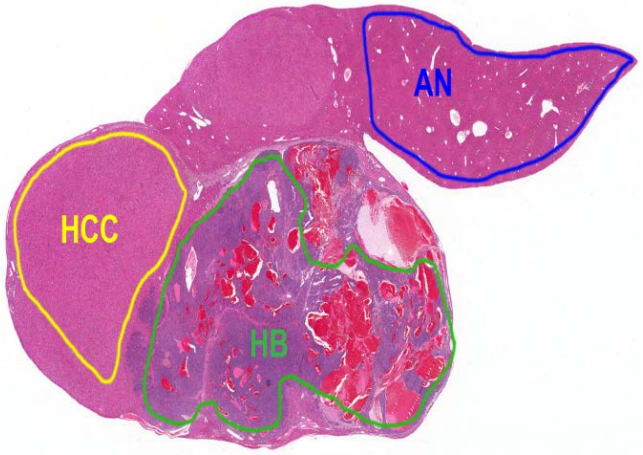
Focus of Cellular Alteration, Atypical (Liver)





These are atypical because of the marked cytomegaly, karyomegaly and cellular atypia but still have features of a focus with a discrete circumscribed, noncompressive cell population that blends into the surrounding hepatic parenchyma

Molecular analysis of paired Hepatoblastoma and adjacent Hepatocellular carcinoma resulting from 2-year BDCA exposure in B6C3F1/N mice



- Hepatocellular Carcinoma (HCC), Hepatoblastoma (HB), and Adjacent Normal (AN) tissues were laser capture microdissected from cryosections (for microarray) or FFPE sections (for mutation analysis)

Samples	*Hras (Codon 61)*		*Ctnnb1 (exon 2*	
	HCC	HB	HCC	HB
Historical controls	260/473* (55%)	NA	1/59# (2%)	NA
BDCA-exposed	4/30 (13%)	2/30 (7%)	3/30 (10%)	7/30 (23%)

* *Incidence of Hras mutations in spontaneous HCC of B6C3F1 mice (Maronpot et al., 1995; Sills et al., 1999)*
\# *Incidence of Ctnnb1 mutations in spontaneous HCC of B6C3F1 mice (Hayashi et al., 2003)*

- Microarray analysis of HB indicated dysregulation of Wnt/Ctnnb1 and embryonic development pathways

Incidence of Neoplastic and Nonneoplastic lesions in the Harderian Gland of Male B6C3F1/N Mice

	Vehicle control	250 mg/L	500 mg/L	1000 mg/L
Epithelium, Hyperplasia[a]	1 (2.0)[a]	1 (1.0)	3 (2.3)	4 (2.0)
Adenoma	6	11	14*	19*
Carcinoma	0	0	0	3
Adenoma or Carcinoma	6*	11	14*	20*

* Significantly different (P≤0.05) from the control group by the Poly-3 test; when in controls, indicates significant trend
[a] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Nonneoplastic lesions in B6C3F1/N Mice exposed to Bromodichloroacetic Acid

- Males
  - Testes – Atrophy at 500 and 1,000 mg/L
  - Epididymis
    - Atrophy – all exposure groups
    - Hypospermia – high exposure group only
    - Epithelium, Degeneration – at 500 and 1,000 mg/L

Summary of Carcinogenic Effects of Bromodichloracetic Acid in B6C3F1/N Mice

- Male B6C3F1/N mice
- ***Clear evidence of carcinogenic activity***:
  - Increased incidences of hepatocellular carcinoma and hepatoblastoma
  - Increased incidences of adenoma or carcinoma (combined) of the Harderian gland
-
- Female B6C3F1/N mice
- ***Clear evidence of carcinogenic activity***:
  - Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma